02-11-05 IFW RCE

Mail Stop RCE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Chuan Li
7908 Avenida Navidad, #158
San Diego, CA 92122
February 10, 2005

Dear Sir or Madam:

This is a re-submission of Request for Continued Examination (RCE). The original RCE was submitted on January 18, 2005. The applicant received a Notice of Non-Compliant Amendment which is enclosed with this letter. The re-submitted RCE includes:
A. A complete listing of all claims
B. The listing of claims includes the text of all pending claims (including withdrawn claims).

The original RCE was submitted according to OG Notices published on February 25, 2003 which is enclosed with this letter. This is the newest OG Notices regarding the amendments to the best of the applicant's knowledge. The formats relating listing and text of cancelled and withdrawn claims are highlighted on pages 3 and 5 of the OG Notice. Should the original RCE be correct, please use the original submitted RCE. Otherwise please use the newly submitted RCE with this letter.

Sincerely,

Chuan Li

Chuan Li

The transmittal form for RCE and fee were submitted on January 18, 2005.
Application Number: 10/068,664
Filing Date: February 6, 2002
First Named Inventor: Chuan Li
Art Unit: 1636
Examiner Name: James S. Ketter

Preliminary Amendments to DE NOVO SYNTHESIZED PLASMID, METHODS OF MAKING AND USE THEREOF

Applicant Name: Chuan Li Chuan Li

Date: January 18, 2005

Application/Control Number: 10/068,664

Art Unit: 1636

**a.) Introductory Comments**

This is a continuation of application (Application Number: 10/068,664) filed on February 6, 2002. The new claims are being submitted as part of this CPA; these claims are submitted to be patentable over the art of record in the parent cases for the following reasons.

Most recombinant plasmids are made by Boyer and Cohn's teaching (US patent number 4,237,224). This conventional method uses a whole existing plasmid as starting material. The plasmids made by this method are modifications of the existing plasmid. Boyer and Cohn fail to teach that a new plasmid can be made without using a whole existing plasmid as starting material.

Stemmer et al synthesized oligos to assemble a plasmid. These oligos are synthesized according to the sequence and structure (organization of plasmid elements such as replication origin and selection marker) of an existing plasmid pUC182Sfi. Their synthesized plasmid is same as the existing plasmid pUC182Sfi. Stemmer et al fail to teach that a new plasmid can be made without referring entire structure of an existing plasmid.

The disclosed application synthesized plasmids with plasmid elements without using any whole existing plasmid as starting material or referring the entire structure of any existing plasmid. Sequences of unknown or undesirable functions are eliminated during plasmid element synthesis. The synthesized plasmid contains only sequences of known and desirable functions. The entire sequences and structure of an existing plasmid is not used

in the synthesis of the novel plasmid. Using plasmid elements from pACYC177, pACYC184, pBR322, and pUC19, the disclosed application synthesized four replication origins and four selection marker genes. From these replication origins and selection marker genes, the disclosed invention synthesized 60 novel plasmids (Example 1 and Example 2 of the disclosed application). Ten of these plasmids were used for future plasmid construction (sequence ID NOS: 32-41). All these plasmids have novel sequences and structures. Many of them also have unexpected properties which are contrary to prior art teachings.

Now the question is whether it is anticipated by Stemmer et al teaching to make the disclosed plasmid for those skilled in the art at the time the invention was made. The applicant believes the claimed invention is not anticipated by Stemmer et al for those skilled in the art at time the invention was made. The reasons are following:

1. Stemmer et al made an existing plasmid. They did not teach how to make a new plasmid. Boyer and Cohn invented conventional method to make new plasmid. Their method cannot anticipate what novel plasmids may be made. As results, many plasmids were patented using Boyer and Cohn's invention. By analogy, if Stemmer et al teaching can be used to make a new plasmid, this method cannot anticipate what novel plasmid may be made. The disclosed plasmid is synthesized without using these prior art methods and it has novel sequences, structure and sometime displays unexpected properties, therefore it is not anticipated by Stemmer et al teaching.
2. The method for synthesizing existing plasmid by Stemmer et al has been available for about 10 years since its publication. Similar method was published even early (Mandecki, W. et al, A totally synthetic plasmid for general cloning, gene expression and mutagenesis in Escherichia coli. Gene 94 (1992) 103-107) and it was still used relative recently (Smith, H.O. et al, PNAS, 12/23/2003, 15440-15445, 100, 26). Because of obvious advantage of saving cellular energy by eliminating sequences of unknown and undesirable functions with the disclosed plasmid, those skilled in the art surely would have implemented it by now. The fact of lack of implementation for about 10 years indicates the claimed plasmid is not anticipated by Stemmer et al.

3. The disclosed application utilizes a new principle of operation to make the synthesized plasmid. Most plasmids from prior art are synthesized from whole existing plasmids as starting material. Stemmer et al synthesized a same plasmid as the existing plasmid pUC182Sfi. Plasmids synthesized from prior arts either using a whole existing plasmid as starting material or referring the entire structure of an existing plasmid. On the other hand, the disclosed application combines elements of plasmid, neither using a whole existing plasmid as starting material nor referring entire structure of any existing plasmid, to synthesize a new plasmid. With teaching of the application, people can make a plasmid (or any other DNA constructs) from plasmid elements without using an existing plasmid as starting material or referring entire structure of an existing plasmid. The application disclosed a new principle of making a novel plasmid. Therefore the application has blazed a trail, rather than followed one. Using new principle of operation indicates the disclosed invention is not anticipated by Stemmer et al.

4. The disclosed invention used plasmid elements (replication origin and selection marker) from pACYC177, pACYC184, pBR322, and pUC19 to synthesize novel plasmids. The disclosed plasmids eliminate significant DNA sequences of unknown and undesirable functions without loss of capability. For example, p4T is synthesized from replication origin of pACYC177 and selection marker of pBR322 (EXAMPLE 1). The disclosed plasmid p4T eliminates DNA sequences with unknown and/or undesirable function. The resulting plasmid p4T (2315 base pairs) is significant smaller than either pACYC177 (3941 base pairs) or pBR322 (4361 base pairs). Other disclosed plasmids all eliminate DNA sequences with unknown and/or undesirable function. They are all smaller than any existing plasmid in the prior arts with same replication origin and selection marker. Plasmid p4T remain the capability of replication and antibiotic selection. As matter of fact, p4T has higher copy number per cell than either pACYC177 or pBR322 and it has higher tetracycline tolerance than pBR322. The omission of significant DNA sequences in the disclosed plasmid without loss of capability indicates the disclosed invention is anticipated by Stemmer et al. The plasmid made by Stemmer's teaching has same sequences as their referred plasmid pUC182Sfi.

5. The disclosed plasmids have unexpected properties. For example, the replication origin of p4T is generated from low copy number plasmid pACYC177; its predicted copy number should be lower than plasmids synthesized from pBR322 and significant lower than the plasmids synthesized from pUC19 according to the teaching from prior arts. However the observed copy number of p4T is higher than or comparable with the plasmids synthesized from pUC19 origin (p3A, Fig. 2) and significant higher than those synthesized from pBR322 origin (p1A, Fig. 2). This unexpected result is contradictory to relevant teachings in molecular biology (Sambrook et al, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., Vol. 1, page 1.3-1.5 and Table 1.1 page 1.4, 1992) that plasmids with replication origin from pACYC177 are low copy number plasmids. As matter of fact, the disclosed plasmids, that are synthesized from the same replication origin, demonstrate different copy number per cell from low copy number (p2K, Fig.2), intermediate copy number (p4K, Fig. 2), to high copy number (p4T, Fig. 2). These results strongly suggest that the copy number of a plasmid is determined not only by its replication origin but also by its selection marker and the relative structure of the plasmid elements. The different copy numbers of the plasmids generated from pACYC177 replication origin are critical to some of the biomedical applications. The unexpected result of the disclosed plasmid indicates the disclosed invention is not anticipated by any prior art. The plasmid made by Stemmer's teaching has same properties as existing plasmid pUC182Sfi.

6. The products generated from the disclosed plasmids have attained commercial acquiescence. Many products are made from the disclosed plasmids and recently commercialized by Expression Technologies Inc. Some of the products are already purchased and used by laboratories in US. One biotech company licensed one of the cell strains comprising a plasmid generated from a disclosed plasmid p4C (sequence ID NO: 37). The cell strain solved the protein expression problem while the same problem cannot be solved using a competitor's cell strain (a reference letter from the scientist purchased our products is enclosed with the preliminary amendments). The replication origin of p4C is from pACYC177 and the chloramphenicol selection marker of p4C is from pACYC184. However neither pACYC177 nor pACYC184 can

be used to solve the particular protein expression problem because of their low copy number. The commercial acquiescence, which solved the protein expression problem that cannot be solved by the cell strain from an established reagent company, indicates the disclosed invention is patentable.

In conclusion, the disclosed invention uses novel processes to make plasmids. The novel processes involve a new principle of operation. The de novo synthesized plasmid has novel sequences and novel, sometimes unexpected properties. Some of the properties are contrary to the teachings of prior arts. It is useful in various biomedical applications. It achieved commercial acquiescence by solving problems of protein expression for academic and industrial identities. It is neither anticipated nor obvious over Stemmer's teaching or any other prior arts or combination of them. Therefore the re-written claims are submitted that patentable subject matter is clearly present. If the examiner agrees but does not feel that the present claims are technically adequate, applicant respectfully requests that the examiner write acceptable claims pursuant to MPEP 707.07(j).